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An Overview of Current Efforts in Short-Term Carcinogen Testing

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Scientists in the Health and Environmental Review Division (HERD), Office of Toxic Substances of the U.S. Environmental Protection Agency, are examining the feasibility of expanding efforts in short-term carcinogen testing. Three areas for consideration have been defined. These are (1) short-term *in vitro* tests; (2) short-term *in vivo* tests; and (3) tumor markers.

HERD's current efforts in short-term *in vitro* testing are exemplified by the Gene-Tox program. Through a comprehensive system of committees and reviews, the published literature on eukaryotic and prokaryotic *in vitro* and *in vivo* test systems are being examined and analyzed. The suitability of utilizing the various systems in a test battery to identify potential chemical mutagens and carcinogens will be ascertained.

A review of the literature on short-term *in vivo* tests (limited bioassays) and tumor markers is currently being conducted. Correlations will be made between results obtained from these tests and epidemiological information and long-term animal bioassays. The attributes and deficiencies of each test or marker will be examined. Further testing, development, or validation needs will be outlined. The aim of this review is to attempt to expand the prechronic test battery for carcinogenicity in order to provide sufficient information for regulatory decision-making.

Section 5 of the Toxic Substances Control Act (TSCA) requires industry to notify the Environmental Protection Agency (EPA) of its intent to initiate manufacturing or processing of a new chemical, or if an existing chemical will be used in a significantly new way. Section 4 of TSCA gives EPA the authority to require industry to conduct testing when there is evidence that a chemical may present an unreasonable risk to health or the environment, and when there is substantial exposure of humans or the environment to the chemical. Both of these sections of TSCA, therefore, provide for the initiation of detailed reviews on chemicals and their analogs.

The process begins with a thorough evaluation of the published literature and any data supplied by industry. Results from studies being conducted by other government agencies and the National Toxicology Program also are evaluated by EPA scientists. Following this comprehensive review, it may be concluded that sufficient information about the potential carcinogenicity of the chemical is already available in order to make a hazard assessment. On the other hand, it may be concluded that additional testing may be required. If short-term tests are recommended, results from these tests may indicate that no further testing is needed, or that the chemical might be a potential carcinogen and that a definitive long-term animal bioassay should be conducted.

While the long-term animal bioassay is the best available method for the detection of animal carcinogens, it nevertheless is very time-consuming, expensive, and often provides ambiguous results. Clearly, to circumvent these problems, more research is needed in short-term carcinogen testing.

Three distinct areas for research have been identified by scientists at the EPA. These are (1) short-term *in vitro* tests, which are exemplified by the Gene-Tox Program; (2) short-term *in vivo* tests or limited bioassays; and (3) tumor markers. At present, only tests which identify complete carcinogens are being examined, although the applicability of these tests to the identification of tumor promoters may also be investigated. None of the tests in any of these research areas provide direct evidence that a chemical is a carcinogen. However, if used in a pre-

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chronic carcinogenicity screen, results from such tests may yield suggestive, or supportive, evidence for the potential carcinogenicity of a compound, and may help in deciding whether additional long-term animal testing is necessary.

The first area of research that I would like to discuss is the area of short-term *in vitro* tests. Most of you are already familiar with the Gene-Tox Program, whose overall objective is to evaluate systematically the current utility of selected mutagenicity and related assay systems based on the available literature. Those assays which have been selected for comprehensive evaluation are based on gene mutations, chromosomal effects, primary DNA damage, or oncogenic transformation. The evaluations are being conducted by scientists representing academia, industry, and government working through a series of committees to examine the feasibility of using these methods in a prechronic testing battery for carcinogenicity.

The next area I would like to discuss is the short-term *in vivo* test. A comprehensive examination of the literature on limited bioassays is being conducted in order to identify potential short-term *in vivo* tests for carcinogens. This review will include an analysis of the data on each selected bioassay for use as a confirmatory test in the carcinogenicity screen. Data base and other validation needs will be identified including false positives and false negatives. Correlations will be made between results obtained with the *in vivo* tests under consideration and epidemiologic information and long-term animal bioassays.

The initial review identified over 20 different short-term *in vivo* bioassays for consideration. From these, five were selected for in-depth analysis. These include (1) the Sencar mouse-skin tumorigenesis with and without promotion; (2) pulmonary tumor induction in strain A mice; (3) pulmonary tumor induction in newborn mice; (4) mammary tumor induction in female Sprague-Dawley rats; and (5) induction of iron-resistant liver foci in rats.

The in-depth analysis of these various short-term *in vivo* tests will be completed within approximately three months. Following this analysis of the literature, laboratory testing of selected bioassays will begin to fill in the data base gaps and to complete the validation of the selected bioassays as predictive tools for carcinogenicity.

The next area of research that I would like to discuss is our efforts on the potential use of tumor markers for carcinogen screening. Ideally, it would be quite advantageous if a marker could be found in the blood which is specific for malignant neoplasms. Such a marker could have several potential uses. For example, it could be used to detect cancers in humans when the tumors are still small and therefore can be most readily treated. It could also be used to follow patients with cancer who are on chemotherapy. That is, as the tumor regresses, the marker may disappear, signifying that the chemotherapy is working. In fact, several identified markers are being used today in just this way. A third possible use of tumor markers is to monitor workers exposed to potential carcinogens. Should these workers develop cancer, these markers will be elevated in the blood and the tumor may be detected at an early stage of development when, again, treatment is most effective. Finally, a fourth potential use of tumor markers is the early detection of cancer in experimental animals who are exposed to potential carcinogens. It is to this last use that I would like to address the rest of my presentation.

Over 25 tumor markers have been mentioned in the literature. Of these, α-fetoprotein and carcinoembryonic antigen have been studied the most. Most of what is known about these markers is through work conducted in humans and human cancers. The potential applicability of using some of these markers in carcinogen testing in rodents is currently being investigated.

The published literature is being searched for information on cancer markers. The data on each selected marker will be analyzed for use as a confirmatory test in the prechronic carcinogenicity screen. When possible, correlations will be made between the ability of each marker to detect known carcinogens or target-organ specificity. Each marker will be ranked as a possible candidate for use in a prechronic test battery.

Where animal and human data exists, this will be specified and correlations will be made. The attributes and deficiencies of each marker, such as false positives and false negatives, chemical-class or target-organ specificity will be identified. Finally, the markers selected for potential use in the prechronic test battery will be further tested and validated, as necessary.

I would like to give just one example of the potential uses of these tumor markers. Serum levels of α-fetoprotein are elevated during the administration of 2-AAF and during the growth period of a transplanted hepatoma. On removal of the tumor, the marker level goes down. On regrowth of the tumor, the serum level of α-fetoprotein begins to rise again. Concentrations of α-fetoprotein in the serum also increase during injury to the liver, as with carbon tetrachloride, or during hepatectomy or pregnancy. During the testing of potential carcinogens, some of these variables could be controlled. The fact that the concentration of α-fetoprotein increases

during noncarcinogenic toxic injury as well as during the growth of neoplasms shows that this marker only produces suggestive evidence for the potential carcinogenicity of a compound. However, additional research in this area can potentially lead to more specificity among markers.

In summary, scientists at the EPA are examining the feasibility of expanding the efforts in short-term carcinogen testing. These efforts include short-term *in vitro* tests, short-term *in vivo* tests, and tumor markers. It is envisioned that ultimately, the prechronic test battery for potential carcinogens may be expanded to include not only tests based on mutagenic endpoints or cell transformation assays, but also results from short-term *in vivo* tests and tumor markers. Taken together, such tests should supply sufficient information to indicate whether additional definitive long-term animal bioassays are required.